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16. ABSTRACT

Using recently developed cultivation and assay systems, currently available methods for concentrating enteric viruses from drinking water by adsorption to and subsequent elution from microporous filters followed by organic flocculation, were evaluated for their ability to recover hepatitis A virus (HAV). Cell culture-adapted HAV (strain HM-175) in seeded tap water was efficiently adsorbed by both electronegative (Filterite) and electropositive (Virosorb 1MDS) filters at pH and ionic conditions previously used for other enteric viruses. Adsorbed HAV was efficiently eluted from these filters by beef extract eluents at pH 9.5. Eluted HAV was further concentrated efficiently by acid precipitation (organic flocculation of eluents containing beef extract made from powdered but not paste sources. Using optimum adsoprtion conditions for each type of filter, HAV was concentrated >100-fold from samples of seeded tap water with about 50% recovery of the initial infectious virus added to the samples. The ability to recover and quantify HAV in contaminated drinking water with currently available methods should prove useful in further studies to determine the role of drinking water in HAV transmission.

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Evaluation of Methods for Concentrating Hepatitis A Virus from Drinking Water

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By using recently developed cultivation and assay systems, currently available methods for concentrating enteric viruses from drinking water by adsorption to and subsequent elution from microporous filters followed by organic flocculation were evaluated for their ability to recover hepatitis A virus (HAV). Cell culture-adapted HAV (strain HM-175) in seeded tapwater was efficiently adsorbed by both electronegative (Filterite) and electropositive (Virosorb 1MDS) filters at pH and ionic conditions previously used for other enteric viruses. Adsorbed HAV was efficiently eluted from these filters by beef extract eluents at pH 9.5. Eluted HAV was further concentrated efficiently by acid precipitation (organic flocculation) of eluents containing beef extract made from powdered, but not paste, sources. By using optimum adsorption conditions for each type of filter, HAV was concentrated >100-fold from samples of seeded tapwater, with about 50% recovery of the initial infectious virus added to the samples. The ability to recover and quantify HAV in contaminated drinking water with currently available methods should prove useful in further studies to determine the role of drinking water in HAV transmission.

Assessment and control of enteric virus contamination of drinking water requires methods for detecting these viruses in finished drinking water. Such methods are especially needed to investigate outbreaks of waterborne disease, to determine the extent of virus removal and inactivation in pilot and full-scale water treatment processes and systems, to monitor wastewater reclamation and reuse systems, and to do epidemiological studies on relationships between risks of waterborne viral illness and virus levels in drinking water. For the more easily cultivated enteric viruses, such as enteroviruses, reoviruses, and some adenoviruses, a variety of methods for their concentration from water have been developed, evaluated, and subsequently used in field studies (1, 7, 8, 21, 22). However, none of these methods has been evaluated for its ability to recover hepatitis A virus (HAV).

HAV is one of the most important waterborne enteric viruses, and many outbreaks of hepatitis A due to contaminated drinking water have been reported (14). During a waterborne outbreak of viral gastroenteritis and hepatitis A in Georgetown, Tex., enteric viruses were concentrated from samples of the incriminated water by schemes involving adsorption to electropositive Virosorb 1MDS and electronegative Filterite filters and elution with beef extract and then organic flocculation (10). Although low levels of HAV antigen were detected in some concentrated samples by radioimmunoassay (RIA), apparently no attempt was made to demonstrate HAV infectivity in either experimental animals or cell cultures. Because it was a field study, the efficiency of HAV recovery by the concentration procedures employed could not be determined.

Until recently, the lack of convenient laboratory methods for HAV cultivation and assay made it impossible to detect this virus in contaminated water or to study its occurrence, survival, transport, and fate in aquatic environments. With recent progress in methods for HAV cultivation and enumerative assay in cell cultures (6, 9, 13, 17), such studies are now possible.

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In this study widely used concentration methods, involving adsorption to and elution from electronegative and electropositive microporous filters and subsequent organic flocculation, were evaluated for their ability to concentrate HAV from seeded drinking water.

MATERIALS AND METHODS

Viruses, cell cultures, and virus assays. The HM-175 strain of HAV was used in all experiments. This strain of HAV was initially isolated from human stools by experimental infection of marmosets, subsequently propagated in primary African green monkey kidney (AGMK) cell cultures for 10 passages, and then grown in BS-C-1 (continuous, AGMK-derived) cell cultures for six to seven passages (3, 13).

Cell cultures were grown in Eagle minimum essential medium containing 10% fetal bovine serum, 0.015 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer and antibiotics (50 μg of gentamycin and 100 μg of kanamycin per ml), and they were maintained in the same medium containing only 2% fetal bovine serum. For stock virus production, freshly trypsinized BS-C-1 cell suspensions in growth medium (10^6 cells per ml) were infected at a multiplicity of about 0.01 infectious unit per cell. Viruses were adsorbed to suspended cells for 1 to 3 h at 37°C with periodic or continuous mixing. Infected cells were then diluted 10-fold into growth medium in 150-cm^2 tissue culture flasks or 890-cm^2 roller bottles and incubated at 37°C for 3 to 5 weeks. HAV infection was monitored by scraping small quantities of cells from culture flasks and examining them for HAV-specific immunofluorescence (15). HAV was harvested from infected cells when >25% of the cells were strongly positive for immunofluorescence. Because HAV remains largely cell associated, virus was harvested by first scraping the cells into the medium, recovering the cells by low-speed centrifugation, suspending them in 5 to 10 ml of phosphate-buffered saline (pH 7.5) and then extracting HAV from this cell suspension with 5 to 10 ml of chloroform. The HAV-containing phosphate-buffered saline was recovered by low-speed centrifugation to remove cell debris and chloroform.

The cell debris and chloroform material was extracted four to six more times with 5 to 10 ml of phosphate-buffered saline to obtain additional cell-associated virus. Phosphate-buffered saline extracts were pooled, filtered through 0.2-μm-porosity membrane filters, and stored at −70°C as virus stocks for later experimental use. These HAV stocks typically contained about 10^7 infectious units per ml.

For all virus stocks and experimental samples, HAV infectivity was quantified by an enumerative assay procedure known as a radioimmunofocus assay (RIFA) (13). HAV in stock preparations and in some experimental samples was also assayed for antigen by a microtiter solid-phase RIA as described by Purcell et al. (18). The RIFA is analogous to a conventional plaque assay, except that localized areas of virus-infected cells are visualized indirectly by an immune autoradiographic procedure because HAV does not produce discernable cytopathic effects or cell death. In this assay confluent monolayers of BS-C-1 cells in 60-mm-diameter tissue culture dishes with film liners (Falcon no. 3006; Becton Dickinson Labware) are inoculated with 0.2 ml of sample and incubated for 60 to 90 min at 37°C with periodic redistribution of the inoculum for virus adsorption. Inoculated cells are overlaid with 5.0 ml of maintenance medium containing 0.5% agarose and incubated at 37°C for about 2 weeks. Cultures are refed with agarose maintenance medium about 1 week postinoculation. After incubation, the agar overlay is carefully removed, and the cell layers on the film-lined dishes are fixed with acetone for 2 min. Fixed cell layers are inoculated with 1.5 ml of ^{125}I-labeled HAV antiserum (750,000 cpm/ml), incubated at 37°C for 4 h, washed with phosphate-buffered saline to remove excess labeled antibody, and air dried. The dried cell layers on their film liners are then cut out and placed against X-ray film (Eastman Kodak Co.; X-AR5) with intensifier screens for 3 to 5 days at −70°C. Films are developed and foci images of infected cells are counted. HAV concentrations are expressed in terms of radioimmunofocus-forming units.

In cases where samples were assayed by both RIA and RIFA, data for HAV adsorption to and elution from filters and for overall recovery were computed based on each assay, thus providing comparisons of assay methods. In later experiments on HAV concentration only RIFA was done, because it quantifies infectious virus and therefore is most relevant to public health concerns about waterborne HAV. The RIFA is also more sensitive than the RIA. In this laboratory the lower limit of HAV detectability by RIA, which is a positive/negative ratio of 2.1, corresponds to about 1,000 radioimmunofocus units (unpublished results). Based on studies by others, the lower limit of HAV detectability by RIA corresponds to about 7×10^5 virions (20a).

Water. Water for laboratory experiments was finished tapwater from the Chapel Hill municipal water treatment plant in Carrboro, N.C. Finished water is produced from the water of University Lake, a surface impoundment on Morgan Creek, by a conventional scheme consisting of alum coagulation-flocculation, sedimentation, mixed media (anthracite-sand) filtration, and chlorination. The characteristics of this water have been described previously (24, 25). Tapwater was dechlorinated with 50 mg of sodium thiosulfate per liter before use in experiments.

Adsorbent filters. Both electronegative and electropositive adsorbent filter media were evaluated for HAV concentration from water. Electronegative fiber glass-epoxy medium (Duofine, Filterite) was used in two layers (one with 0.45-μm porosity over one with 0.25-μm porosity) as either 25- or 47-mm-diameter disks. Electropositive charge-modified fiber glass medium (Virosorb 1MDS; Cuno Division, AMF Corp.) was used in two layers, each with 0.2 μm porosity, as either 25- or 47-mm-diameter disks. Filter disks were placed in autoclavable plastic or stainless steel filter holders and sterilized by autoclaving.

Eluents. Eluents tested in laboratory experiments were as follows: 0.3% beef extract–50 mM glycine (BE-GLY), 3.0% beef extract–50 mM glycine, 3.0% beef extract, 3.0% beef extract–50 mM glycine–2 M $NaNO_3$ (BE-GLY-$NaNO_3$), and 1.0% nonfat dry milk–50 mM glycine (NFDM-GLY). All eluents were adjusted to pH 9.5 with NaOH. Eluent volumes of 5 and 15 ml were used to elute adsorbed HAV from 25- and 47-mm-diameter filters, respectively. For laboratory studies, beef extract eluents were prepared initially from beef extract paste (Difco Laboratories). In subsequent laboratory experiments two different powdered beef extracts (Lab-Lemco from Oxoid and extract from GIBCO Laboratories) were evaluated for elution and organic flocculation of HAV.

Filter adsorption-elution experiments. Microporous filter methods were evaluated for HAV concentration from seeded tapwater in small-scale experiments. Volumes of dechlorinated tapwater were seeded with stock HAV to give initial virus concentrations of about 10^3 to 10^4 radioimmunofocus-forming units per ml. Samples of seeded water were adjusted to various pH and ionic conditions previously found to be effective for the adsorption of other enteric viruses onto microporous filters. For electronegative Filterite filters, adsorption conditions were pH 3.5 with 0.5 or 5.0 mM $AlCl_3$ and pH 5.5, 6.5, or 7.5 with 5.0 mM $MgCl_2$ (4, 19, 26, 29, 30). For electropositive Virosorb 1MDS filters, adsorption conditions were pH 4.5, 5.5, 6.5, 7.5, or 8.5 with no added divalent cation salts (23). Samples of 50 ml or 1.5 liters of each conditioned water were filtered through 25- or 47-mm-diameter filters, respectively, at flow rates of about 2 ml/min per cm^2 of filter surface area. Filtrates were collected and sampled for assay of unadsorbed viruses. Virus adsorption efficiencies were computed from the differences in virus concentrations between the seeded water before filtration and the filtrates. Adsorbed viruses were eluted from filters by dropwise collection of previously indicated eluent fluids. Eluates were adjusted to pH 7.3 with 0.1 N HCl and assayed for eluted viruses. HAV elution efficiencies were determined from the total amount of virus in the eluate compared to the total amount of virus adsorbed to the filter. Overall HAV recovery efficiencies were determined from the total amount of virus in the eluate compared with the total amount of virus in the volume of seeded water before conditioning and filtration.

To compare the effectiveness of different eluents or sources of beef extract for HAV elution efficiency, HAV in specified volumes of seeded tapwater was adsorbed to a series of replicate filters under a given set of conditions. Filtrates were collected and assayed for unadsorbed viruses. Adsorbed HAV was then eluted from each filter with one of the test eluents, and the eluates were assayed. HAV elution and recovery efficiencies were computed as described above.

Organic flocculation experiments. HAV concentration by organic flocculation of beef extract and nonfat dry milk was evaluated (2, 12). In these experiments, 7-ml volumes of test eluents were seeded with stock HAV to give an initial concentration of about 10^3 radioimmunofocus-forming units per ml. Eluents were adjusted to desired pH levels with 0.1 or 1.0 N HCl and mixed slowly for 20 min. Samples were centrifuged at 3,000 × *g* for 20 min to sediment the floc that

TABLE 1. HAV concentration from 50-ml volumes of seeded tapwater by adsorption to and elution from 25-mm-diameter filters[a]

Filter type	Adsorption conditions	Initial HAV (%)					
		Adsorbed		Eluted[b]		Recovered	
		RIFA	RIA	RIFA	RIA	RIFA	RIA
Filterite	pH 3.5, 0.5 mM $AlCl_3$	>99	94	13	32	13	30
	pH 3.5, 5.0 mM $AlCl_3$	80	83	72	72	58	60
	pH 5.5, 5.0 mM $MgCl_2$	92	91	85	88	78	80
	pH 6.5, 5.0 mM $MgCl_2$	22	33	45	88	10	29
	pH 7.5, 5.0 mM $MgCl_2$	10	15	>99	60	11	9
Virosorb 1MDS	pH 4.5	>99	90	41	96	41	86
	pH 5.5	98	86	69	>99	68	95
	pH 6.5	97[c]	80	46	48	47	38
	pH 7.5	82	68	49	51	40	35
	pH 8.5	43	40	26	30	11	12

[a] Mean results of four experiments.
[b] Elution with 5 ml of BE-GLY (pH 9.5).
[c] Mean results of two experiments only.

formed. Supernatants were decanted and sampled for assay of unadsorbed HAV. The sedimented floc was suspended in 0.1 M Na_2HPO_4 to 1/10 the original sample volume, adjusted to pH 7.3, and assayed for recovered HAV.

RESULTS

HAV adsorption to and elution from microporous filters. The mean results of four replicate experiments on HAV concentration from 50-ml volumes of seeded tapwater by adsorption to and elution from 25-mm-diameter electronegative Filterite and electropositive Virosorb 1MDS filters are summarized in Table 1. Filterite filters adsorbed HAV with ≥80% efficiency at pH 3.5 with 0.5 or 5.0 mM $AlCl_3$ and at pH 5.5 with 5.0 mM $MgCl_2$. Maximum HAV adsorption of >99% occurred at pH 3.5 with 0.5 mM $AlCl_3$. HAV adsorption was relatively poor at pH 6.5 and 7.5 with 5.0 mM $MgCl_2$. HAV was efficiently eluted (≥72%) from Filterite filters by pH 9.5 BE-GLY when adsorption was at pH 3.5 with 5.0 mM $AlCl_3$ or at pH 5.5 with 5.0 mM $MgCl_2$. Under these conditions, overall HAV recoveries were ≥58%. In these experiments HAV elution efficiencies were somewhat lower when adsorption was at pH 3.5 with only 0.5 mM $AlCl_3$. Although HAV that was adsorbed at pH 6.5 and 7.5 with 5.0 mM $MgCl_2$ was eluted efficiently, overall virus recoveries were poor due to inefficient adsorption to filters.

Virosorb 1MDS filters adsorbed HAV with ≥97% efficiency at pH 4.5, 5.5, and 6.5. Adsorption efficiency was somewhat lower (82%) at pH 7.5 and considerably lower (43%) at pH 8.5. Decreased virus adsorption at higher pH levels by Virosorb 1MDS filters has been previously observed for other enteroviruses such as poliovirus (23). Based on RIFA results, HAV was eluted most efficiently (69%) from Virosorb 1MDS filters by pH 9.5 BE-GLY when adsorbed at pH 5.5. However, elution efficiencies were still ≥41% when HAV was adsorbed at pH 4.5, 6.5, or 7.5. Overall HAV recovery was maximum (68% by RIFA) when adsorption was at pH 5.5, but recoveries were still ≥40% at adsorption pH levels of 4.5, 6.5, and 7.5. Under their optimum conditions, both types of filters achieved similar HAV recoveries.

Further experiments on microporous filter methods were done with 47-mm-diameter filters and 1.5-liter volumes of HAV-seeded tapwater to model hydraulic loading conditions more typical of filter use under field conditions. Processing 1.5 liters of water through a 47-mm-diameter filter is equivalent to processing about 400 liters of water through a 10-in. (ca. 25-cm)-long pleated cartridge filter. The mean results for six replicate experiments with Filterite filters (Table 2) show that HAV was adsorbed efficiently (≥93%) at pH 3.5 with 0.5 or 5.0 mM $AlCl_3$ and at pH 5.5 with 5.0 mM $MgCl_2$. Adsorption efficiency was only 43% at pH 6.5 with 5.0 mM $MgCl_2$. Elution of adsorbed HAV was most efficient (66%)

TABLE 2. HAV concentration from 1.5-liter volumes of seeded tapwater by adsorption to and elution from 47-mm-diameter filters

Filter type	Adsorption conditions	Initial HAV (%)[a]		
		Adsorbed	Eluted[b]	Recovered[c]
Filterite	pH 3.5, 0.5 mM $AlCl_3$	97 (5.9)	46 (6.8)	46 (11)
	pH 3.5, 5.0 mM $AlCl_3$	93 (2.6)	66 (9.0)	65 (6.4)
	pH 5.5, 5.0 mM $MgCl_2$	94 (3.9)	52 (2.4)	49 (2.8)
	pH 6.5, 5.0 mM $MgCl_2$	43 (5.3)	44 (4.9)	19 (9.1)
Virosorb 1MDS	pH 4.5	98 (1.6)	72 (3.1)	70 (1.4)
	pH 5.5	97 (3.7)	53 (3.0)	51 (3.3)
	pH 6.5	88 (5.1)	54 (9.0)	49 (6.6)
	pH 7.5	93 (2.8)	47 (3.0)	43 (2.6)

[a] HAV was assayed by RIFA; shown are mean results of six replicate experiments with Filterite filters and seven replicate experiments with Virosorb 1MDS filters; values within parentheses are standard deviations.
[b] Elution with 15 ml of 3% beef extract–50 mM glycine (pH 9.5).
[c] Recovery was based on initial HAV titer in seeded tapwater, before salt additions or pH adjustments.

TABLE 3. Elution of HAV from 25-mm-diameter filters[a]

Filter type	Eluent (5 ml, pH 9.5)	Initial HAV adsorbed (%)		Adsorbed HAV recovered (%)		Initial HAV recovered (%)[b]	
		RIFA	RIA	RIFA	RIA	RIFA	RIA
Filterite	BE-GLY	94	96	55	62	52	60
	3.0% Beef extract	88	95	68	61	60	58
	BE-GLY-$NaNO_3$	85	94	77	62	65	58
	NFDM-GLY	88	92	59	13	52	12
Virosorb 1MDS	BE-GLY	65	49	38	58	25	28
	3.0% Beef extract	57	42	54	70	31	29
	BE-GLY-$NaNO_3$	58	56	69	77	40	43
	NFDM-GLY	69	53	12	5	9	3

[a] HAV was adsorbed from 50-ml volumes of seeded tapwater. Adsorption conditions: Filterite, pH 5.5, 5.0 mM $MgCl_2$; Virosorb 1MDS, pH 7.5. Values are means of duplicate trails.

[b] Calculations were based on initial HAV titer in seeded tapwater, before salt additions or pH adjustments.

when adsorption was at pH 3.5 with 5.0 mM $AlCl_3$, but elution efficiencies of ≥44% were obtained for HAV adsorbed under other conditions. Overall HAV recovery was also most efficient when adsorption was at pH 3.5 with 5.0 mM $AlCl_3$. Recovery for this adsorption condition was significantly greater than recoveries for the other adsorption conditions tested ($P < 0.05$ in two-sample t tests). However, HAV recoveries of 46 and 49% were obtained for HAV adsorbed at pH 3.5 with 0.5 mM $AlCl_3$ and at pH 5.5 with 5.0 mM $MgCl_2$, respectively. For adsorption at pH 6.5 with 5.0 mM $MgCl_2$, the HAV recovery of only 19% was significantly lower than recoveries for the other adsorption conditions tested ($P < 0.05$ in two-sample t tests).

In seven replicate experiments, adsorption of HAV to Virosorb 1MDS filters was ≥88% for all pH levels tested, but the maximum adsorption of 97 and 98% was obtained at the two lowest pH levels, pH 4.5 and 5.5, respectively (Table 2). Elution of adsorbed HAV was maximum (72%) when adsorption was at pH 4.5, but elution efficiencies of 47 to 54% were obtained for HAV adsorbed at other pH levels. Overall HAV recovery was also maximum (70%) when adsorption was at pH 4.5; this recovery was significantly greater than recoveries for the other adsorption pH levels tested ($P < 0.05$ in two-sample t tests). However, HAV recoveries of 43 to 51% were obtained at the other adsorption pH levels used.

HAV recoveries by Filterite and Virosorb 1MDS filters under their optimum conditions (pH 3.5 with 5.0 mM $AlCl_3$ and pH 4.5, respectively) were not significantly different ($P < 0.05$ in two-sample t tests). The results of these experiments on HAV adsorption to and elution from electronegative and electropositive microporous filters demonstrate that HAV can be concentrated from tapwater by using adsorption conditions and a beef extract-glycine eluent previously used successfully for concentrating other enteroviruses. Under optimum conditions, HAV adsorption, elution, and overall recovery efficiencies are comparable to those obtained in previous studies on poliovirus and other enteroviruses (23, 26, 27).

Elution of adsorbed HAV by different eluents. In the experiments described above, adsorbed HAV was eluted from filters with pH 9.5 BE-GLY. Because other eluents containing beef extract or substitute materials and additives have been used previously for virus elution from filters and other surfaces (2, 11, 12, 28), four different eluents were compared for their ability to elute adsorbed HAV from 25-mm-diameter Filterite and Virosorb 1MDS filters. Eluents tested were BE-GLY, 3% beef extract, BE-GLY-$NaNO_3$, and NFDM-GLY, all at pH 9.5.

Mean results of duplicate experiments in which HAV was assayed by RIA and RIFA are summarized in Table 3. Note that the results of both assay methods generally agree, except for NFDM-GLY with Filterite filters. The lower HAV antigen recovery efficiencies from Filterite based on RIA data may be due to some type of interference of the nonfat dry milk with the microtiter solid-phase RIA procedure. All four eluents were effective in eluting HAV from Filterite filters, but the highest elution efficiency (77% based on RIFA) was obtained with BE-GLY-$NaNO_3$. With this eluent, overall HAV recovery was also highest (65% based on RIFA). HAV adsorbed to Virosorb 1MDS filters was also eluted most efficiently (69% based on RIFA) by BE-GLY-$NaNO_3$, but elution efficiency with just 3% beef extract was 54% by RIFA. However, HAV elution efficiency from Virosorb 1MDS was only 38% (by RIFA) with BE-GLY and only 12% (by RIFA) with NFDM-GLY. Overall HAV recovery efficiency from Virosorb 1MDS filters was also highest (40% by RIFA) with BE-GLY-$NaNO_3$. For all eluents, HAV recovery efficiencies with Virosorb 1MDS were lower than those with Filterite, primarily because of lower adsorption efficiencies under the conditions used for the former filter. HAV was adsorbed to Virosorb 1MDS filters at pH 7.5 in these and all subsequent experiments because these filters are typically used in the field with no pH adjustment of sample waters near neutral pH. HAV was adsorbed to Filterite filters from tapwater adjusted to pH 5.5 and supplemented with 5.0 mM $MgCl_2$ in this and all subsequent experiments because divalent cation salts at less acidic pH levels are effective in enhancing virus adsorption, are widely used in both laboratory and field studies, and require less drastic pH adjustment than that required for $AlCl_3$ (19, 26, 29, 30).

The results of these experiments demonstrate that beef extract eluents are capable of eluting HAV from both Filterite and Virosorb 1MDS filters and that elution efficiency is further improved by the addition of $NaNO_3$, a weak chaotropic agent. Nonfat dry milk was effective in eluting HAV from Filterite filters, but not from Virosorb 1MDS filters.

Concentration of HAV by beef extract organic flocculation. Beef extract and beef extract-glycine are the two most widely used eluents for recovering viruses adsorbed to microporous filters, and viruses eluted with these materials are often further concentrated by organic flocculation of the beef extract eluate (1, 2, 7, 8). For these reasons a series of experiments was done to determine optimum pH conditions for HAV concentration from 3% beef extract eluent (Difco

TABLE 4. Organic flocculation of HAV from paste beef extract eluent at different pH levels[a]

Flocculation pH	Initial HAV (%) recovered in:			
	Supernatant		Floc	
	RIFA	RIA	RIFA	RIA
3.0	64	43	28	29
3.5	54	38	30	31
4.0	66	51	18	24
4.5	52	45	48	44
5.0	77	63	23	28

[a] Flocculation from 7-ml volumes of 3% beef extract (Difco). Mean results of triplicate experiments.

Laboratories paste) by organic flocculation. As shown by the summarized results of triplicate experiments (Table 4), considerable amounts of HAV (52 to 77%) did not flocculate and remained in the supernatant after centrifugation at flocculation pH levels between 3.0 and 5.0. Maximum recovery of HAV (48% by RIFA) was obtained by flocculation at pH 4.5, and recoveries of about 30% were achieved by flocculation at pH 3.0 and 3.5. The results of these experiments indicate that HAV recovery efficiencies of no more than 50% are achieved by organic flocculation of 3% beef extract made from Difco paste at pH 4.5, because about one-half of the total initial virus is not flocculated.

Because of the relatively poor flocculation and recovery of HAV by beef extract made from Difco paste, further experiments on organic flocculation of HAV were done with two different sources of powdered beef extract (Oxoid and GIBCO). As shown by the mean results of four replicate experiments (Table 5), both sources of powdered beef extract flocculated HAV more efficiently than did the paste beef extract previously tested. With Oxoid beef extract, maximum HAV recoveries in the floc and minimum residual HAV in the supernatant were obtained at pH 3.0, 3.5, and 4.0. HAV recoveries at these pH levels were significantly greater than those at pH 4.5 or 5.0 ($P < 0.05$ in two-sample t tests). With GIBCO beef extract, maximum HAV recoveries in the floc were at pH 3.5 and 4.0, but minimum residual HAV in the supernatant was obtained at pH 3.0 as well as at pH 3.5 and 4.0. HAV recoveries at pH 3.5 and 4.0 were significantly greater than recoveries at the other pH levels tested ($P < 0.05$ in two-sample t tests). At each pH level, HAV recoveries for the two powdered beef extracts were not significantly different ($P < 0.05$ in two-sample t tests). The results of these experiments indicate that HAV can be concentrated more efficiently by organic flocculation of beef extract made from powdered sources than with beef extract from paste sources.

TABLE 5. Organic flocculation of HAV from powdered beef extract eluents at different pH levels[a]

Flocculation pH	Initial HAV (%) recovered in:			
	Supernatant		Resuspended floc	
	Oxoid	GIBCO	Oxoid	GIBCO
3.0	1.1 (0.2)	2.2 (0.9)	60 (17)	37 (10)
3.5	1.1 (0.2)	2.2 (0.4)	56 (26)	58 (17)
4.0	1.5 (0.2)	4.4 (3.5)	54 (22)	62 (15)
4.5	11 (7.5)	47 (23)	39 (10)	35 (28)
5.0	69 (36)	68 (31)	9.4 (5.7)	7.9 (1.9)

[a] Flocculation from 7-ml volumes of 3% beef extract–50 mM glycine; mean results of four replicate experiments; values within parentheses are standard deviations.

TABLE 6. Elution and recovery of HAV from microporous filters by different sources of beef extract[a]

Filter type	Beef Extract	Initial HAV adsorbed (%)	Adsorbed HAV eluted (%)	Initial HAV recovered (%)
Virosorb 1MDS	Oxoid	81 (9.1)	51 (16)	42 (14)
	GIBCO	86 (12)	54 (35)	47 (31)
	Difco	86 (8.4)	54 (37)	47 (30)
Filterite	Oxoid	88 (15)	91 (8.5)	83 (21)
	GIBCO	88 (10)	96 (6.4)	96 (6.4)
	Difco	91 (4.0)	92 (13)	91 (15)

[a] Filter diameter, 47 mm; sample volume, 1.5 liters; eluate, 15 ml of 3% beef extract–50 mM glycine (pH 9.5); adsorption conditions, pH 7.5 for Virosorb 1MDS and pH 5.5 with 5.0 mM added $MgCl_2$ for Filterite. Shown are mean results of four replicate experiments with Virosorb 1MDS and three replicate experiments with Filterite; values within parentheses are standard deviations.

Comparison of beef extracts for elution and recovery of adsorbed HAV. Previous experiments on elution and recovery of HAV adsorbed to microporous filters were done with beef extract made from paste (Difco) (Tables 1 and 2). Because this beef extract was found to be a poor flocculant for HAV, a series of experiments on elution and recovery of HAV adsorbed to Filterite and Virosorb 1MDS filters was done with 3% beef extract–50 mM glycine (pH 9.5), made from two powdered sources (Oxoid and Gibco) and well as paste (Difco). HAV in 1.5-liter volumes of dechlorinated tapwater was adsorbed to Virosorb 1MDS filters at pH 7.5 and to Filterite filters at pH 5.5 and 5.0 mM added $MgCl_2$.

As shown by the summarized results of replicate experiments (Table 6), all three sources of beef extract gave similar elution efficiencies for the same filter type; these elution efficiencies were not significantly different ($P < 0.05$ in two-sample t tests). It is interesting to note, however, that under the adsorption conditions used, HAV elution efficiencies were significantly greater from Filterite than from Virosorb 1MDS filters for all three beef extracts ($P < 0.05$ in a two-sample t test). The results of these experiments demonstrate that two powdered beef extracts are comparable to a paste beef extract for HAV elution from both Filterite and Virosorb 1MDS filters.

DISCUSSION

Because of the epidemiological importance of HAV as a waterborne virus, existing methods for enteric virus concentration from water involving adsorption to and elution from microporous filters and organic flocculation were evaluated for their ability to concentrate a laboratory-adapted strain of HAV from seeded tapwater. Results of laboratory studies with small volumes of seeded tapwater indicated that HAV can be concentrated by adsorption to and elution from electronegative and electropositive microporous filters previously used for concentrating other enteric viruses. HAV was efficiently adsorbed by electronegative Filterite filters at pH 3.5 with added $AlCl_3$ and at pH 5.5 with added $MgCl_2$. HAV was adsorbed most efficiently by electropositive Virosorb 1MDS filters at pH 4.5 and 5.5 without added multivalent cations, and adsorption was still relatively efficient at pH 7.5. Adsorbed HAV was eluted most efficiently from both types of filters with BE-GLY-$NaNO_3$ at pH 9.5.

Elution also was relatively efficient with other beef extract eluents. Overall HAV recovery efficiencies by these conventional microporous filter methods were similar to those previously obtained for other enteric viruses (23, 27).

The observation that the weak chaotrope $NaNO_3$ in combination with alkaline beef extract was more effective than other eluents containing beef extract or nonfat dry milk suggests that both electrostatic and hydrophobic interactions are probably involved in HAV adsorption to microporous filter media. This finding is consistent with those of previous studies demonstrating that both types of reactions are involved in the adsorption of other enteric viruses to microporous filters and estuarine sediments (5, 20, 28).

Initial laboratory experiments on HAV concentration from beef extract (Difco paste) eluents by organic flocculation at acidic pH levels showed that maximum recovery efficiencies of about 50% could be achieved at pH 4.5. The unrecovered virus was not flocculated (precipitated) and remained in the resulting supernatant. This phenomenon of incomplete flocculation has been reported previously for other enteric viruses, such as echoviruses 1 and 7, coxsackieviruses A9 and B2 through B5, and minute virus of mice (2, 16, 26, 28; F. P. Williams, Jr., and W. Jakubowski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q35, p. 200). In subsequent organic flocculation experiments with two powdered sources of beef extract (Oxoid and Gibco), HAV flocculation and recovery efficiencies were considerably higher than those of previous experiments with beef extract made from paste. For powdered beef extracts, maximum HAV recoveries of >50% occurred at pH 3.5 and 4.0, and they were also efficient with one powdered source at pH 3.0. These findings are consistent with those of Hurst et al. (11), who reported that organic flocculation of poliovirus type 1, echovirus type 7, and coxsackievirus A9 was more efficient with a number of powdered beef extracts, including the two of this present study, than with Difco paste beef extract. When Difco paste and the two powdered beef extracts were compared for their ability to elute HAV adsorbed to Filterite and Virosorb 1MDS filters, all three gave similar elution efficiencies for the same type of filter. However, additional research is needed on elution and organic flocculation methods to further improve the recoveries of HAV and other enteric viruses.

The results of this study demonstrate that HAV can be concentrated from contaminated drinking water by using current methods employing electronegative and electropositive microporous filters and organic flocculation of beef extract eluents. Although HAV recoveries were greater with Filterite filters when adsorbing at pH 5.5 and 5.0 mM $MgCl_2$ than with Virosorb 1MDS filters when adsorbing at pH 7.5 (Table 6), the latter filter still achieved HAV recoveries of nearly 50%. Virosorb 1MDS filters are more convenient for field use because conditioning of the sample water is not necessary if the pH does not exceed 7.5. In fact, in this laboratory we have used Virosorb 1MDS filters with no conditioning of sample waters to successfully detect and quantify HAV in four of six samples of contaminated drinking water from a ground water source during a hepatitis A outbreak in a small, rural community in western Maryland (M. D. Sobsey, S. E. Oglesbee, and D. A. Wait, Water Sci. Technol., in press).

The ability to concentrate and detect HAV in drinking water with conventional microporous filter and organic flocculation methods should prove useful in further research efforts to assess and control HAV contamination of drinking water. However, additional studies are needed to evaluate these methods and other methods for HAV recovery from large volumes of drinking water and from natural waters and wastewaters as well.

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LITERATURE CITED

1. **American Public Health Association.** 1985. Detection of enteric viruses, section 913, p. 946–974. *In* Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, D.C.
2. **Bitton, G., B. N. Feldberg, and S. R. Farrah.** 1979. Concentration of enteroviruses from seawater and tapwater by organic flocculation using non-fat dry milk and casein. Water Air Soil Pollut. 12:187–195.
3. **Daemer, R. J., S. M. Feinstone, I. D. Gust, and R. H. Purcell.** 1981. Propagation of human hepatitis A virus in African green monkey kidney cell culture: primary isolation and serial passage. Infect. Immun. 32:388–393.
4. **Farrah, S. R., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Concentration of viruses from large volumes of tapwater using pleated membrane filters. Appl. Environ. Microbiol. 31: 221–226.
5. **Farrah, S. R., D. O. Shah, and L. O. Ingram.** 1981. Effect of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters. Proc. Natl. Acad. Sci. USA 78:1229–1232.
6. **Froesner, G. G., F. Deinhardt, R. Scheid, V. Gauss-Muller, N. Holmes, V. Messelberger, G. Siegl, and J. J. Alexander.** 1979. Propagation of human hepatitis A virus in a hepatoma cell line. Infection 7:303–305.
7. **Gerba, C. P.** 1983. Methods for recovering viruses from the water environment, p. 19–35. *In* G. Berg (ed.), Viral pollution of the environment. CRC Press, Boca Raton, Fla.
8. **Goyal, S. M., and C. P. Gerba.** 1982. Concentration of viruses from water by membrane filters, p. 59–116. *In* C. P. Gerba and S. M. Goyal (ed.), Methods in environmental virology. Marcel Dekker, Inc., New York.
9. **Gust, I. A., S. A. Locarnini, A. Coulepis, and K. von der Helm.** 1982. The biology of hepatitis A virus, p. 35–56. *In* F. Deindardt and J. Deinhardt (ed.), Viral hepatitis: laboratory and clinical science. Marcel Dekker, Inc., New York.
10. **Hejkal, T. W., B. Keswick, R. L. LaBelle, C. P. Gerba, Y. Sanchez, G. Dreesman, B. Hafkin, and J. L. Melnick.** 1982. Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. J. Am. Water Works Assoc. 74:318–321.
11. **Hurst, C. J., D. R. Dahling, R. S. Safferman, T. Goyke, and B. A. Wright.** 1984. Comparative evaluation of commercial beef extracts and similar materials for recovering viruses from environmental samples. Can. J. Microbiol. 30:1253–1263.
12. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentraton method for the detection of viruses in tap water. Appl. Environ. Microbiol. 32:638–639.
13. **Lemon, S. M., L. N. Binn, and R. H. Marchwicki.** 1983. Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures. J. Clin. Microbiol. 17:834–839.
14. **Lippy, E. C., and S. C. Waltrip.** 1984. Waterborne disease outbreaks—1946–1980: a thirty-five-year perspective. J. Am. Water Works Assoc. 76:60–67.
15. **Mathiesen, L. R., S. M. Feinstone, R. H. Purcell, and J. Wagner.** 1977. Detection of hepatitis A antigen by immunofluorescence. Infect. Immun. 18:524–530.
16. **Morris, R., and W. M. Waite.** 1980. Evaluation of procedures for recovery of viruses from water. II. Concentration systems. Water Res. 14:795–798.
17. **Provost, P. J., and M. R. Hilleman.** 1979. Propagation of human

hepatitis A virus in cell culture in vitro. Proc. Soc. Exp. Biol. Med. 160:213–221.
18. **Purcell, R. H., D. C. Wong, Y. Moritsugu, J. L. Dienstag, J. A. Routenberg, and J. D. and Boggs.** 1976. A microtitier solid-phase radioimmunoassay for hepatitis A antigen and antibody. J. Immunol. 116:349–356.
19. **Rao, N. U., and N. A. Labzoffsky.** 1969. A simple method for the detection of low concentrations of viruses in large volumes of water by the membrane filter technic. Can. J. Microbiol. 15:399–403.
20. **Shields, P. A., and S. R. Farrah.** 1983. Influence of salts on electrostatic interactions between poliovirus and membrane filters. Appl. Environ. Microbiol. 45:526–531.
20a. **Simmonds, R. S., G. Szücs, T. G. Metcalf, and J. L. Melnick.** 1985. Persistently infected cultures as a source of hepatitis A virus. Appl. Environ. Microbiol. 49:749–755.
21. **Sobsey, M. D.** 1976. Methods for detecting enteric viruses in water and wastewater. p. 89–127. *In* G. Berg, H. L. Bodily, E. H. Lennette, J. L. Melnick, and T. G. Metcalf (ed.), Viruses in water. American Public Health Association, Washington, D.C.
22. **Sobsey, M. D.** 1982. Quality of currently available methodology for monitoring viruses in the environment. Environ. Int. 7:39–51.
23. **Sobsey, M. D., and J. S. Glass.** 1980. Poliovirus concentration from tap water with electropositive adsorbent filters. Appl. Environ. Microbiol. 40:201–210.
24. **Sobsey, M. D., and J. S. Glass.** 1984. Influence of water quality on enteric virus concentration by microporous filter methods. Appl. Environ. Microbiol. 47:956–960.
25. **Sobsey, M. D., J. S. Glass, R. J. Carrick, and W. A. Rutala.** 1980. Evaluation of the tentative standard method for enteric virus concentration from large volumes of tap water. J. Am. Water Works Assoc. 72:292–299.
26. **Sobsey, M. D., J. S. Glass, R. R. Jacobs, and W. A. Rutala.** 1980. Modifications of the tentative standard method for improved virus recovery efficiency. J. Am. Water Works Assoc. 72: 350–355.
27. **Sobsey, M. D., J. S. Glass, and R. S. Moore.** 1981. Evaluating adsorbent filter performance for enteric virus concentrations in tap water. J. Am. Water Works Assoc. 73:542–548.
28. **Wait, D. A., and M. D. Sobsey.** 1983. A method for the recovery of enteric viruses from estuarine sediments using chaotropic agents. Appl. Environ. Microbiol. 46:379–385.
29. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. Apparatus for concentrating viruses from large volumes, J. Am. Water Works Assoc. 64:189–196.
30. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. A portable virus concentrator for testing water in the field. Water Res. 6:1249–1256.

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